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Antiproliferative Effect of Suramin on Primary Cultures of Human Pheochromocytomas and Rat PC12 Pheochromocytoma Cells

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Background: Suramin inhibits growth of neural crest-derived cells and is used to treat adrenocortical cancer and neuroblastoma in clinical trials. The antiproliferative effect of suramin was evaluated in primary cultures of human pheochromocytoma and the PC12 rat pheochromocytoma cell line in vitro and in vivo.

Methods: Human pheochromocytoma and PC12 rat pheochromocytoma cells were grown in medium supplemented with suramin at concentrations of 1–1,000 μg/ml (1.43–1.43 mM) for up to five generations. Suramin did not induce neuronal differentiation, but inhibited growth of cultured human pheochromocytoma cells with IC_{50} (inhibitory concentration at which a 50% reduction of proliferation is observed) of 50–250 μg/ml. Also, suramin inhibited proliferation of PC12 cells with IC_{50} of 228 μg/ml after 5 days and 161 μg/ml at 10 days of treatment. Colony formation assays demonstrated these effects to be cytotoxic rather than cytostatic. Thus when reproductive integrity of PC12 cells was taken into account, IC_{50} was calculated with 118 μg/ml and 129 μg/ml, respectively. In vivo experiments were performed with subcutaneously xenotransplanted PC12 cells (BALB/c NCR-NU mice). Suramin did not alter tumorigenicity and did not inhibit local tumor growth.

Results: These data determine for the first time an antiproliferative effect of suramin in pheochromocytoma cells. Suramin is cytotoxic to pheochromocytoma cells in vitro at levels that are clinically achievable.

Conclusions: Suramin may have potential as an antiproliferative drug in nonresectable pheochromocytoma.

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KEY WORDS: suramin; human pheochromocytoma; cell culture; PC 12 cells; antiproliferative drug usage

INTRODUCTION

Suramin is a polysulfonated naphtylurea initially used in the treatment of onchocerciasis and Rhodesian trypanosomiasis. Since its recent recognition as a potent inhibitor of DNA polymerases and reverse transcriptase, it has undergone a considerable amount of investigation and has been used in treating acquired immunodeficiency

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syndrome (AIDS), although with limited success [1]. However, such studies made investigators aware of suramin's antitumor activity and resulted in formal investigations of suramin as an antitumor drug [1,2]. Several mechanisms of antiproliferative action have been established, including inhibition of a wide range of cytoplasmatic and membrane-bound enzyme systems [3], nonselective interference with the binding to cellular receptors by growth factors [1,4], and interfering with tumor cell adhesion, migration, and invasion [5,6]. Suramin induces cell differentiation in colon carcinoma, neuroblastoma and rhabdomyosarcoma cells [6–8]. Antiproliferative activity is established for neuroblastoma, glioblastoma, adrenocortical cancer, and osteosarcoma, as well as colon, renal, and prostate cancer [1].

The most effective treatment for pheochromocytoma is surgical excision. However, for metastatic disease or unresectable tumors, currently used protocols of radiotherapy and chemotherapy are only partially effective. 131 I-labeled mataiodobenzylguanidine (MIBG) has been used since 1984 [9,10]. Although an improvement of clinical symptoms is reported by most patients, only 30–50% display hormonal response [11]. Among these, a few experience reduction of tumor volumes and only occasionally is lasting tumor response observed [12,13]. Moreover, only 60% of patients with malignant pheochromocytoma show MIBG uptake [10]. Chemotherapy with a triple combination of cyclophosphamide, vincristine, and darcabazine will have some evidence of tumor response in 60% of patients, the great majority being partial remissions [14]. Biochemical response is documented in nearly 80% of patients treated, although the benefit of such response may be debatable, since improved pharmacological control of catecholamine secretion does not influence mortality or tumor survival [15]. Since suramin is cytotoxic to various neural crest-derived cells and is used in clinical trials to treat advanced stages of adrenocortical cancer and neuroblastoma [1,16], we were interested in evaluating the antiproliferative effect of suramin in primary cultures of human pheochromocytoma in vitro and in the PC12 rat pheochromocytoma cell line in vitro and in vivo.

MATERIALS AND METHODS

Chemicals

Suramin, provided by the Center of Disease Control (Atlanta, GA), was prepared as sterile stock solution in 0.15 M NaCl and stored at −20°C. Prior to the experiments, the solution was thawed, filtered with suramin-saturated filters, and diluted appropriately. Suramin media, as well as preparations for in vivo experiments, were used within 30 minutes after preparation. Dimethylthiazol-dephenyltetrazolium bromide (MTT) and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Heat-inactivated fetal calf serum (FCS) was purchased from Hy-Clone Lab (Logan, UT) and equine serum (ES) obtained from GIBCO Labs (Grand Island, NY). All other cell culture reagents were purchased from the Cell Culture Facility, University of California Medical Center (San Francisco).

Cells and Culture Conditions

Primary cell cultures of human pheochromocytoma were prepared from surgically removed pheochromocytomas following techniques described in detail elsewhere [17–19]. Samples were transported in cold alpha minimal essential medium (MEM) supplemented with 10% FCS, penicillin (50 IU/ml), and streptomycin (50 μg/ml). The pheochromocytoma tissue was freed from adjacent stromal tissue using a dissection microscope and minced until a tissue homogenate was obtained. The homogenate was subjected to enzymatic digestion using collagenase type I (1 mg/ml PBS) and trypsin (125 μg/ml PBS) for appropriate time intervals. The resultant suspension was filtered using a 200 μm mesh, resuspended in growth medium, and seeded. Cells were incubated at 37°C, 5% CO_2, 100% humidity for up to 24 hours. The loosely adherent pheochromocytoma cells were dislodged by forceful trituration and transferred to flasks precoated with poly-ornithin (0.1 mg/15 mM borate buffer). Early passages were used for experiments only.

The PC12 cell line is a clonal line of rat adrenal pheochromocytoma established in 1976, which has been extensively characterized [19,20]. PC12 cells were obtained from American Tissue Culture Collection (Rockville, MA) and maintained in RPMI-1640, 10% ES, and 5% FCS (penicillin, 50 IU/ml, streptomycin, 50 μg/ml) at 37°C, 5% CO_2, and 100% humidity. Only flasks appearing healthy by morphology and showing >95% viability by tryphan blue exclusion were used for experiments. During the experiments, antibiotics were omitted from growth media.

Drug Sensitivity In Vitro

Proliferation assay. Cellular drug sensitivities were measured utilizing the MTT-colorimetric cell quantitation assay as described previously [21]. Cells were harvested with 0.25% cold trypsin and single cell suspensions confirmed by light microscopy. Human pheochromocytoma cells were seeded in 100 μl tissue culture medium at 5,000 cells per well and PC12 cells at a density of 20,000 cells per well into six wells of poly-ornithin pretreated 96-well microtitre plates. Cells were allowed to adhere and resume full growth pattern under standard conditions. After 48 hours, complete growth medium containing suramin (1–1,000 μg/ml = 1.43 μM–1.43 mM) was added to achieve a final volume of 200 μl. Cells were cultured for up to five generation times with renewal of medium every 48 hours. Cell numbers were determined every 24–48 hours by colorimetric

cell quantitation from triplicate wells on an ELISA microplate reader. Cell number and viability were also assessed by tryphan blue exclusion from triplicate wells. Likewise, continuous monitoring of cell loss to supernatant was obtained by evaluating aspirates from each change of medium for nonadherent, viable floating cells. Experiments were done twice for human pheochromocytoma cultures and in quadruplicate for PC12 cells. The antiproliferative effect is expressed as IC_{50}, which indicates the inhibitory concentration of the drug, resulting in a 50% reduction of proliferation when compared to untreated cells.

Clonogenic assay. Since proliferation assays do not always allow differentiation between cytostatic effects and cytotoxic effects, colony formation assays were performed to evaluate the drug's effect on the reproductive integrity of pheochromocytoma cells. These experiments were done in PC12 cells. Plates and cells were prepared as above. After 5 and 10 days of exposure to the respective concentrations of suramin, PC12 cells were harvested and 2,000 viable cells of each drug concentration were plated into duplicate 60 mm Petri dishes and cultured for an additional 10 days under standard conditions in drug-free medium. Colonies were fixed and counted. Cytotoxicity was assessed by counting colonies of 32 cells and more as positive (i.e., intact reproductive integrity). Results of two replicate plates from duplicate experiments are expressed in absolute numbers of colonies counted per dish [17]. Similar assays were performed with viable cells floating in the supernatant (nonattached cells). Plating efficiency under these conditions must be expected to be in the order of 100–200 colonies for PC12 cells.

Drug Sensitivity In Vivo

Pathogen-free BALB/c NCR-NU congenitally athymic female mice, 4–6 weeks old, were obtained from Simonsen Laboratories (Gilroy, CA). The mice were housed in sterilized cages on laminar air flow benches at 24°C and a 12-hour light/dark cycle. Animals were fed an autoclaved standard breeding chow and water ad libitum and were allowed to adapt to the laboratory environment for at least 7 days prior to any manipulation. They were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL.) as needed. All procedures involving animals were monitored and approved by the local ethics committee. The mice were inoculated subcutaneously with 1×10^6 to 1×10^7 human pheochromocytoma cells at various sites; however, no tumor growth was observed. The addition of reconstituted basement membrane (Matrigel) or various other purified extra cellular matrix proteins also did not result in local tumor growth. In contrast, inoculation of $0.5–3 \times 10^6$ PC12 cells resulted in growth of solid tumors with a tumor take rate of 100% and reproducible tumor doubling times. Therefore, PC12 cells were used for evaluation of in vivo drug sensitivity.

In these experiments, 3×10^6 PC12 cells were inoculated subcutaneously to the flank of 5–6-week-old female BALB/c NCR-NU-mice. In order to evaluate tumor volumes reliably and reproducibly, tumors were allowed to grow for 18 days until they reached an average size of 30 cubic mm. Mice were then randomly allocated into treatment and control groups of five mice each and received either 2 mg suramin dissolved in 100 μl of 0.15 M NaCl (~350 mg suramin/m^2/mouse and day) intraperitoneally, or 100 μl 0.15 M NaCl alone. The application scheme followed that of a recently published study [22]. Suramin was administered on days 18,19,20,23,26, and every 6 days thereafter. To determine whether co-application of suramin at the time of xenotransplantation would alter tumorigenicity of PC12 cells, injections were started immediately after inoculation of tumor cells and randomization of mice in a second experiment. These animals were randomized into groups of 10 mice each and given either 100 μl of 2 mg suramin/ml 0.15 M NaCl intraperitoneally, or 100 μl 0.15 M NaCl alone. Suramin was administered on days 1,2,3,6,9, and every 6 days thereafter. Again, groups of five mice each served as control groups: tumor (receiving no treatment at all); suramin (tumor-free mice receiving suramin); and untreated (no suramin, no tumor). Every 3 days, body weights were taken and general well-being assessed by the presence or absence of any signs of drug-related toxicity. Tumors were measured in three dimensions using calibrated micrometer calipers and tumor volumes calculated with $(1 \times w \times h) \times 0.5236$, where 1 is length, w width, and h height [23]. Drug treatment was discontinued at 39 days and 33 days after inoculation of cells, for experiments 1 and 2, respectively. Mice were anesthetized, sacrificed by cervical dislocation, exsanguinated, and assessed for local tumor growth, macroinvasion, and macrometastases. Body weight and wet weights of tumors and various viscera were taken.

Statistical Analysis

IC_{50} values were computed using standard linear regression analysis (Sigma Plot for Windows, San Rafael, CA). Likewise, tumor dimensions were used to compute tumor volume doubling times. Paired serial observations of days postinoculation (x) and corresponding log tumor volume (y) at the respective time points were individually entered for each mouse/tumor as multiple separate pairs of conjugate variables. Best-fitting curve equations were obtained by standard regression analysis and individual tumor doubling times calculated by dividing log 2 by the slope of the regression line determination for each tumor. Significance of differences between tumor volumes in treated vs. control groups was computed using two-tailed rank-sum tests. Levels of significance of tu-

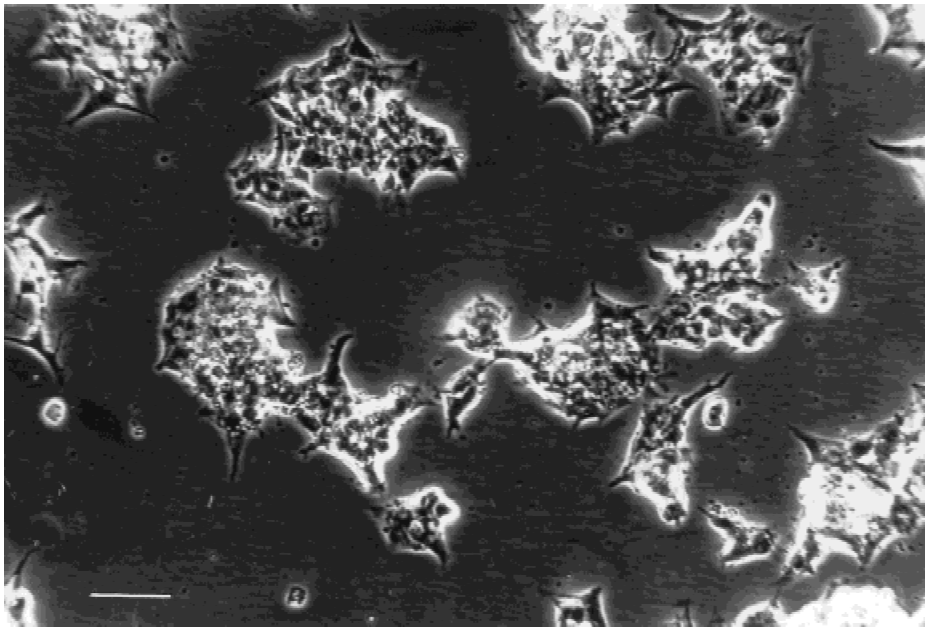

Fig. 1. Representative photomicrograph of human pheochromocytoma cells grown on poly-ornithin precoated culture dishes. Cells display a monomorphous population of polygonal shaped cells of regular size. Cells are firmly attached to the tissue culture dish (magnification ×160, bar:100 μm).

mor volume doubling times and weights were tested by the two-tailed Student *t*-test. $P < 0.05$ was considered a significant difference. Values are given in mean ± standard deviation (SD), if not stated otherwise.

RESULTS

Growth Inhibition of Human Pheochromocytoma Cell Cultures

Primary cultures of human pheochromocytomas were established from seven surgically removed pheochromocytomas. A representative photomicrograph of a human pheochromocytoma in culture is given in Figure 1. Five of the cell lines could be maintained in culture for sufficient time periods to allow proliferation assays to be performed at least twice. Data from these primary cultures were used to determine the antiproliferative effects of suramin in vitro.

The efficacy of suramin's antiproliferative and cytotoxic effect was clearly dose-dependent in the different cell lines. During proliferation assays, a greater than 50% reduction of growth was always achieved with concentrations of 250 μg/ml. Clearly, cytotoxic effects of suramin were observed in four out of the five cultures, with concentrations ranging from 250 μg to 500 μg/ml suramin. The calculated IC_{50} for suramin in human pheochromocytomas varied considerably and ranged from 50 μg to 250 μg/ml suramin (Table I). Only one pheochromocytoma cell culture (HPC 6) revealed a fraction of surviving cells after exposure to the maximum concentration studied (lethal dose >1,000 μg/ml suramin).

TABLE I. Effect of Suramin on Proliferation of Primary Cultures of Human Pheochromocytomas

Cell line	IC_{50}[a]		LD[b]
HPC 1	50	100	250
HPC 3	75	100	250
HPC 5	100	250	500
HPC 6	160	250	>1,000
HPr 2	75	100	250

[a]Numbers indicate the concentration of suramin (μg/ml) at which 50% inhibition of proliferation is observed (IC_{50}, calculated as in Materials and Methods). Since two experiments were performed, both repeats are presented.

[b]LD (lethal dose) represents the concentration of suramin at which no remaining viable cells were observed.

Growth inhibition of human pheochromocytoma cells was due to a cytotoxic rather than cytostatic effect, since cell viability as assessed by tryphan blue exclusion yielded results similar to those of the MTT assay. Exposure of the cells to rising concentrations of the drug did not result in acquisition of a differentiated phenotype. On the contrary, dying cells revealed a morphology reminiscent of apoptosis, displaying protrusion of cytoplasm, and enlargement of nuclei, as well as condensation and fragmentation of chromatin.

Growth Inhibition of PC12 Pheochromocytoma Cells

When PC12 rat pheochromocytoma cells were studied, comparable results were obtained. Suramin inhibited growth of PC12 cells in a dose-dependent manner as

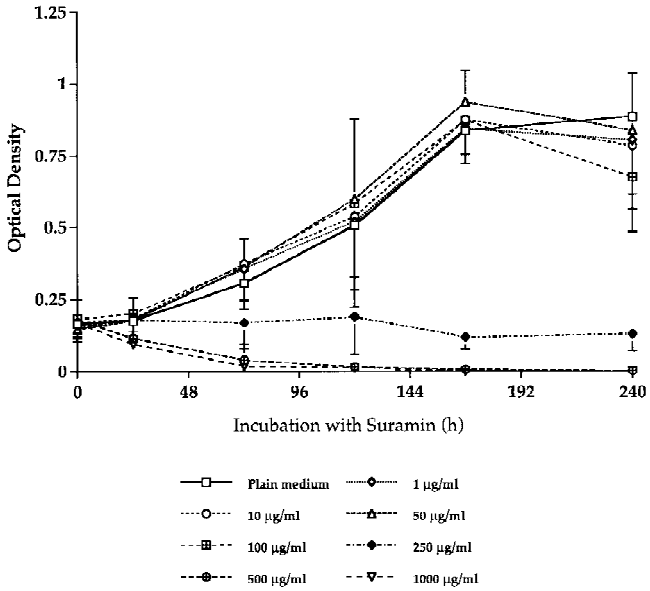

Fig. 2. Suramin dose response of PC12 pheochromocytoma cells in vitro. Cell numbers were measured by colorimetric cell quantitation (MTT assay) at the indicated times. The data illustrated as optical densities represent means ± SD of four independent experiments in triplicate. Highest optical densities, although not statistically significant, were obtained from PC12 cells grown in the presence of suramin at 1–100 μg/ml. Growth inhibition by 50% occurred with 228 μg suramin/ml during log phase at 120 hours and 161 μg/ml during late log phase after 240 hours of exposure to the drug.

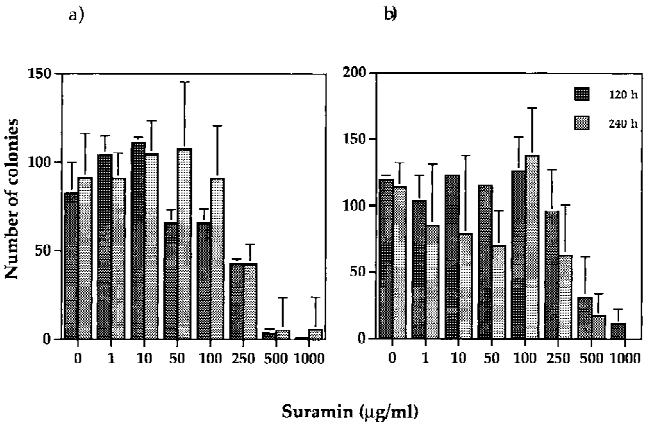

Fig. 3. **(a)** The effect of suramin on reproductive integrity of PC12 pheochromocytoma cells as determined by cologenic assay. PC12 cells were exposed to suramin for 120–240 hours at the indicated concentrations. Cells from each drug concentration were then incubated for 10 additional days without suramin. Reproductive integrity was defined as the ability of cells to grow colonies of >32 cells and is expressed as absolute numbers of colonies counted/dish. **(b)** Similar assay performed as in (a) from viable cells floating in the supernatant. Means ± SD for two independent experiments in duplicate.

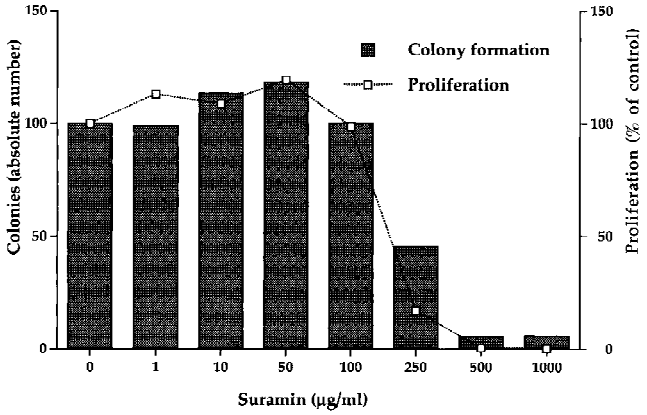

Fig. 4. Overlay of dose response curves as determined from proliferation and colony formation assays. Values are given as a percentage of surviving cells as compared to controls (points) and in absolute number of colonies (columns) after 240 hours of exposure to suramin. Colony formation is expressed as the mean of at least two independent experiments in duplicate. Proliferation is presented as the mean of four independent experiments in triplicate. Standard deviation did not exceed 20% in both assays, respectively. Identical results were obtained for 120 hours of exposure to suramin. Both curves are similar, indicating cytotoxicity.

early as 24 hours following addition of the drug (Fig. 2). A small but reproducible stimulation of cell proliferation was observed for low concentrations of suramin (1–100 μg/ml), corroborating the findings of others that suramin at sublethal doses appears to stimulate proliferation [16,24]. However, >50% inhibition of growth was demonstrated with suramin concentrations of >250 μg/ml. At high concentrations of suramin, only a few cells remained viable, and those were less likely to adhere to the dishes. As monitored by continuous evaluation of aspirates from each change of medium, the total amount of viable cells lost to the supernatant was always <3%. IC_{50} was calculated with 228 μg/ml at 5 days and 161 μg/ml at 10 days of treatment, respectively. To determine whether these effects were cytotoxic rather than cytostatic, colony formation assays were performed. Suramin also impaired the ability of PC12 cells to reproduce in vitro. Since floating cells were noted at concentrations of suramin >250 μg/ml, it was necessary to perform colony formation assays for both attached and floating viable cells. The results obtained were essentially identical (Fig. 3). Moreover, the dose response curve from the colony formation assays closely resembled that of the proliferation assays with comparable IC_{50}, thus providing conclusive evidence that suramin is cytotoxic rather than cytostatic to pheochromocytoma cells (Fig. 4). Therefore, IC_{50} values from the proliferation assays were corrected for colony formation ability in order to identify the reproductive fraction of cells, i.e., the number of cells that had maintained their reproductive integrity. For example, of the 35% of PC12 cells that survived 5 days of growth in 250 μg suramin/ml, only 58% were able to form colonies, giving a ''true survival rate,'' i.e., reproductive fraction, of 20.3%. IC_{50} values for the reproductive fraction of PC12 cells were calculated with 118 and 129 μg/ml at 5 days and 10 days of treatment, respectively. Differentiation of PC12 cells is characterized by cessation of cell growth, increase in cell size as well as adhe-

TABLE II. Effect of Suramin on Xenotransplanted PC12 Cells*

	Experiment 1			Experiment 2		
Condition	n	Tumor volume[a]	Doubling time[b]	Tumor volume	Tumor weight[c]	Doubling time
PC12 + suramin	(5/10)	444 ± 159[d]	4.17	422 ± 189	440 ± 120	4.21
PC12 + NaCl	(5/10)	435 ± 73	4.25	399 ± 196	389 ± 168	4.33
PC12 no treatment	(5/5)	457 ± 149	4.20	442 ± 148	468 ± 67	4.28

*Xenografts grew for 39 days (experiment 1) and either 350 mg suramin/m2/dose or 100 μl 0.15M NaCl were administered intraperitoneally on days 18, 19, 20, 23, 26, and every 6 days thereafter. In experiment 2, injections were started at inoculation of PC12 cells and tumor growth was observed for 33 days. Numbers in parentheses indicate number of mice per group and experiment.
[a]Tumor volumes (cubic mm).
[b]Tumor volume doubling times (days).
[c]Tumor wet weights (mg) of PC12 cells inoculated to BALB/c NCR-NU-mice.
[d]means ± standard deviation.

sion to tissue culture dishes, and acquisition of a neuronal phenotype by formation of axon-like processes [19,20]. Such changes were not observed; instead the morphologic changes were similar to those observed in human pheochromocytoma cells. Thus suramin did not induce phenotypic differentiation of human or rat PC12 pheochromocytoma cells.

Growth of Xenografted PC12 Cells

Since human pheochromocytoma cells could not be established as xenografts, the PC12 cell line was used to study the potential of suramin to inhibit proliferation in vivo. PC12 cells grew as xenografts in BALB/c NCR-NU-mice with a tumorigenicity of 100% and reproducible tumor volume doubling times. Application of suramin failed to inhibit growth of xenografted PC12 cells when treatment was started after tumor growth had already been established. In a separate study, application of suramin was started immediately upon inoculation of tumor cells, which did not result in a decrease of tumorigenicity. Also, no effect of suramin on growth of PC12 cells was observed, despite apparent signs of drug-related toxicity (Table II). Untreated controls reached a mean body weight of 22.1 ± 1.0 g, and mice with xenografted PC12 tumors not receiving any treatment reached 23.4 ± 2.1 g. Suramin-treated mice bearing PC12 xenografts rarely grew >20 g (mean 16.9 ± 1.6 g, $P < 0.05$) as did tumor-free control mice receiving the drug (19.1 ± 1.9 g, $P < 0.05$). Likewise, wet weights of a number of viscera were the lowest in suramin-treated mice (data not shown). In both trials, impaired general well-being of suramin-treated, tumor-bearing mice was the reason for discontinuing the experiment.

DISCUSSION

The observation that suramin appeared to alter the growth of human pheochromocytoma cells in vitro instead of inducing differentiation prompted our interest to evaluate the antiproliferative effect of suramin in pheochromocytoma. We found suramin effectively to inhibit growth of human pheochromocytoma as well as PC12 rat pheochromocytoma cells at pharmacological doses in vitro. Signs of neuronal differentiation were not observed during the experiments, indicating that suramin, contrary to its effects in colonic and other neuronal cell lines, does not induce phenotypic differentiation of pheochromocytoma cells in vitro [4,7,8]. The results indicate that human pheochromocytoma and PC12 cells are highly sensitive to suramin. IC_{50} values for PC12 rat pheochromocytoma cells appeared to be somewhat higher than for human pheochromocytomas, but they compare favorably when reproductive integrity is taken into account. Such concentrations are readily achieved in humans with acceptable toxicity. Pheochromocytomas exhibit a chemosensitivity to the drug similar to that of the N2A neuroblastoma cell line, glioblastoma, osteosarcoma, and adrenocortical cancer cell lines [16,17,24–27]. Suramin was shown to inhibit proliferation by interfering with ligand receptor-mediated mitogenic pathways, specifically the growth-promoting effect of insulin-like growth factors [27,28]. Suramin inhibits binding of IGF I and IGF II to its receptors in neuroblastoma and rhabdomyosarcoma cells [27–29]. Increased levels of IGFs have been documented in a variety of neuroendocrine tissues, including human neuroblastoma, pheochromocytoma, and PC12 cells [30,31].

Pheochromocytoma cells are capable of de novo synthesis of IGFs and IGF binding proteins, and IGFs appear to be the dominant autocrine growth factors in neuroendocrine tissues and PC12 cells [31–33]. Thus an obvious possibility of the antiproliferative effect observed in pheochromocytoma in vitro would be the interference with such autocrine loops. But one must take into account also that suramin has pleiotropic effects, not limited to the interaction between growth factors and their receptor, but extended to other metabolic pathways. Among these, the ability of suramin to compete with triphosphates, especially ATP, for their binding sites ap-

pears to play a critical role in the activity and toxicity of suramin [34]. Such competitive inhibition was suggested as a mechanism of cellular toxicity in the DU 145 prostate cancer cell line. Suramin competitively and reversibly blocks binding of ATP to P2-purinoceptor in PC12 cells [35] and ATP-evoked catecholamine secretion [36]. However, the underlying mechanisms of suramin's cytotoxic effect on pheochromocytoma cells in vitro remain to be elucidated.

In vivo, suramin treatment did not result in modulation of tumor growth or tumor take rates of xenografted PC12 rat pheochromocytoma cells. There are several reasons to explain this finding. First, this is a rather preliminary description of growth of xenografted PC12 cells. At present, the characteristics of this model are only marginally understood, and it is unclear whether PC12 cells xenografted to nude mice constitute a reliable in vivo model of pheochromocytoma. Second, we are not aware of the concentrations and retention time of suramin in PC12 xenografts. One may consider the dose and schedule used in these experiments to be suboptimal for obtaining effective concentrations of the drug in PC12 tumor tissue. Moreover, the in vivo antiproliferative effect of suramin may have been attenuated by its binding to serum albumin. Therefore, whereas antiproliferative effects are observed in vitro at concentrations similar to those used in clinical trials, the concentration of free drug can be considerably lower in vivo [37]. Although growth of human osteosarcoma xenografts in nude mice was markedly inhibited when treated with a regimen similar to the one used in the present study [22], established models of xenografted pheochromocytomas do not exist, making a conclusive comparison difficult. Clearly, continued investigations are necessary further to determine the utility of PC12 xenografts as an in vivo model of pheochromocytoma. However, the present study demonstrates for the first time that suramin reproducibly inhibits proliferation of pheochromocytoma cells in vitro. Suramin was cytotoxic to human and PC12 rat pheochromocytoma cells in vitro at concentrations that are clinically achievable. Suramin concentrations of up to 300 μg/ml are readily obtained with acceptable toxicity [16]. Therefore, suramin may have potential as an antitumor agent in pheochromocytoma.

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